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A 40-Year Journey in Search of Selective Antiviral Chemotherapy*

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*François-Rabelais University of Tours (France) is celebrating its 40th anniversary. As this article covers a 40-year journey in the field of antiviral research, I am honored to dedicate this paper to Tours to commemorate both 40-year anniversaries.

Keywords

interferon, poly(I).poly(C), BVDU, acyclovir, NRTIs, NtRTIs, ANPs, NNRTIs, HAART

Abstract

My search for a selective antiviral chemotherapy started more than 40 years ago with interferon inducers, then shifted to nucleoside analogs with the discovery of BVDU (brivudin), a highly selective anti-HSV-1 and anti-VZV agent, and to dideoxynucleoside analogs such as d4T (stavudine), anti-HIV agents. The search culminated in the discovery of acyclic nucleoside phosphonates (ANPs) (in collaboration with Antonin Holý), a key class of compounds active against HIV, hepatitis B virus, and DNA viruses at large; the best known of these compounds is tenofovir. Along the way, the principle of the non-nucleoside reverse transcriptase inhibitors (NNRTIs) was established. This work, initiated in collaboration with the late Paul A.J. Janssen, eventually led to the identification of rilpivirine as perhaps an “ideal” NNRTI.

PRELUDE: FROM LEUVEN TO STANFORD AND BACK TO LEUVEN

This review is the second I have written for the Annual Reviews series; the first was the one I wrote with Thomas C. Merigan on interferon and interferon induction exactly 40 years ago (1). From the title of this 1970 review (1), it is rather obvious that I started my career studying interferon. This was not my choice but that of my mentor, Piet De Somer, professor of microbiology and immunology at the University of Leuven. At the oral examinations following both my fourth and fifth years of medical school studies (1963 and 1964), he asked me whether I would be interested in coming to work in his laboratory. I told him that I was not interested in bacteria, viruses, or the like, but only in biochemistry. (I was at that time working on a diagnostic test for the chemical analysis of catecholamines in the Laboratory of Chimie Hormonologique in the French-speaking part of our university.) Professor De Somer, not used to refusals from students, replied that I could work on the (bio)chemistry of viruses. I was not totally convinced but nevertheless accepted his offer, so that in August 1966, one month after I had graduated as an MD from the University of Leuven (Louvain at the time), I started to work on interferon in Professor De Somer's laboratory. In a few months' time, I made two observations that I thought were not that important but that my boss found most exciting: (*a*) the fact that interferon could be detected in the urine of rabbits (2) (which meant interferon really existed and that its molecular weight fell below the threshold for kidney passage); and (*b*), more importantly in my eyes, the fact that interferon could be induced by synthetic polyanions, such as polyacrylic acid and polymethacrylic acid. The latter observation would later be the subject of two articles in the second volume of the *Journal of Virology* (3, 4). (Professor De Somer was listed as first author according to custom at that time, but my name appearing second to his was enough of an honor for me.)

After he returned from the Pan American Health Organization (PAHO) meeting where he had presented our data on the urinary excretion of interferon in rabbits (2), Professor De Somer told me I should study with someone in the United States to get some exposure to the American way of life and research. He gave me three options: Tom Merigan at Stanford University (Palo Alto, California), Bob Wagner at Johns Hopkins University (Baltimore, Maryland), or Phil Marcus at Albert Einstein College of Medicine of Yeshiva University (Bronx, New York). The decision on where to go was not difficult: On September 3, 1968, two days after we married, my wife Lili and I flew from Brussels (via London, Los Angeles, and San Francisco) to Palo Alto (the last part by helicopter). We were greeted on the evening of our arrival at Hyatt Rickey's Hotel (which no longer exists) by Dr. Joan Merigan, Tom's wife, who told us that a new life was waiting for us at Stanford. Joan was right. Stanford was a totally new adventure. In no time, Lili and I got acquainted with our new environment. In the next two years (our original plan was to stay for only one year), I would publish as coauthor with my Stanford mentor, Tom Merigan, approximately 20 papers in a number of prestigious journals including *Nature* (5, 6), *Science* (7), the *Journal of Clinical Investigation* (8), and the *Journal of Molecular Biology* (9). Life at Stanford (1968–1970) was delightful on all levels (academic, scientific, and social), so leaving that heavenly place was not the obvious choice, but by the end of 1970 my wife and I did return to Leuven. At Stanford I followed (without being formally enrolled in) the biochemistry courses of Arthur Kornberg, Paul Berg, and the other famous biochemists of the department. I did not earn a degree at Stanford, but my stay there had a durable impact on my career, which I resumed in Leuven under the guidance of Professor De Somer (10).

INTERFERON: ANOTHER PRELUDE

Before we described the induction of interferon by synthetic polyanions such as polyacrylic acid and polymethacrylic acid (3, 4), Merigan described interferon induction by another synthetic

polyanion, pyran copolymer (11, 12); this obviously facilitated my choice to stay with Tom Merigan at Stanford. Professor De Somer had been fascinated by the discovery of interferon by Isaacs & Lindenmann (13) and believed, albeit initially, that interferon would become the panacea for viral infections, much as another British discovery, penicillin, had proven to be for bacterial infections.

What attracted me personally to interferon was not the molecule itself, which for many years after its discovery in 1957 had remained an esoteric principle rather than a chemically identifiable entity, but the galvanizing observations made by Maurice Hilleman's group at Merck [published in a series of five consecutive *PNAS* papers (all sponsored by Max Tishler) (14–18)] that interferon could be induced by double-stranded RNAs from both viral and synthetic origins. I always viewed the discovery of interferon induction by double-stranded RNAs such as poly(I).poly(C) as the highlight of my early interferon days, and although Maurice Hilleman got his principal recognition for the many vaccines he developed (19), the importance of poly(I).poly(C) as an inducer of interferon should not go unnoticed. Even though poly(I).poly(C) never became licensed as an antiviral medicine, it had a durable impact on the field of host defense to viral infection (20), and it proved essential in our attempts to isolate and determine the structure of human fibroblast interferon (21), to clone and express human fibroblast interferon (22), and to identify interferon β 2 (23). Identification of the latter, later to be renamed interleukin-6 (IL-6), in a certain sense could be considered a spin-off of interferon research. In the same year (1982), I wrote a review article titled “Interferon: a molecule for all seasons” (24). Interferon has indeed been a molecule for all seasons, and for many years as well; with the use of (fibroblast) β -interferon for the treatment of multiple sclerosis and of α -interferon for the treatment of hepatitis C, interferon has become a “never-ending story” (25).

BVDU:

(*E*)-5-(2-bromovinyl)-2'-deoxyuridine, or brivudin

HSV-1: herpes simplex virus type 1

VZV: varicella-zoster virus

BRIVUDIN: A POTENT AND SELECTIVE ANTIHERPESVIRUS AGENT

The story of BVDU [(*E*)-5-(2-bromovinyl)-2'-deoxyuridine, or brivudin], as I have written previously (26, 27), dates back to 1976 (see **Figure 1**). Its potency and selectivity as a herpes simplex virus type 1 (HSV-1) inhibitor was described in 1979 (28), and after we found it to be highly active against varicella-zoster virus (VZV), we initiated a first clinical study—with apparent success—that included oral BVDU treatment in four patients with severe herpes zoster (29). In retrospect, this was a daring initiative, as we did not obtain (or seek) permission from any ethical committee for undertaking such a clinical study (apparently, Professor De Somer's name as senior author on the paper gave it sufficient credibility). Further clinical development of BVDU followed a rocky route. It was originally developed at Searle UK but then dropped by Searle US. (Other companies were not interested because BVDU, albeit much more active than acyclovir against VZV, did not prove—as acyclovir did—markedly active against HSV-2, the principal cause of genital herpes.) Yet BVDU was rescued by Berlin-Chemie (located in the former DDR), and after East and West Germany were united in 1989, Berlin-Chemie was taken over by the Italian company The Menarini Group. Thanks to the efforts of the latter (and particularly the efforts of a certain Antonio Giachetti), BVDU finally became a licensed product for the treatment of herpes zoster at a single daily dose of 125 mg (for 7 days) in many European countries, including Germany (Zostex[®]), Italy (Brivirac[®]), and Belgium (Zerpex[®]).

Inspired by the pioneering work of Herbert Kaufman on the use of idoxuridine (IDU) and trifluridine (TFT) in the topical treatment of HSV keratitis (30) (both compounds are still used for the topical treatment of herpetic eye infections), we described in 1980 in rabbits (31) and shortly thereafter in humans (32) the usefulness of BVDU eye drops (at 0.1%) in the topical treatment of HSV keratitis. BVDU (at 0.1%) proved superior to IDU (0.1%) in the topical treatment of

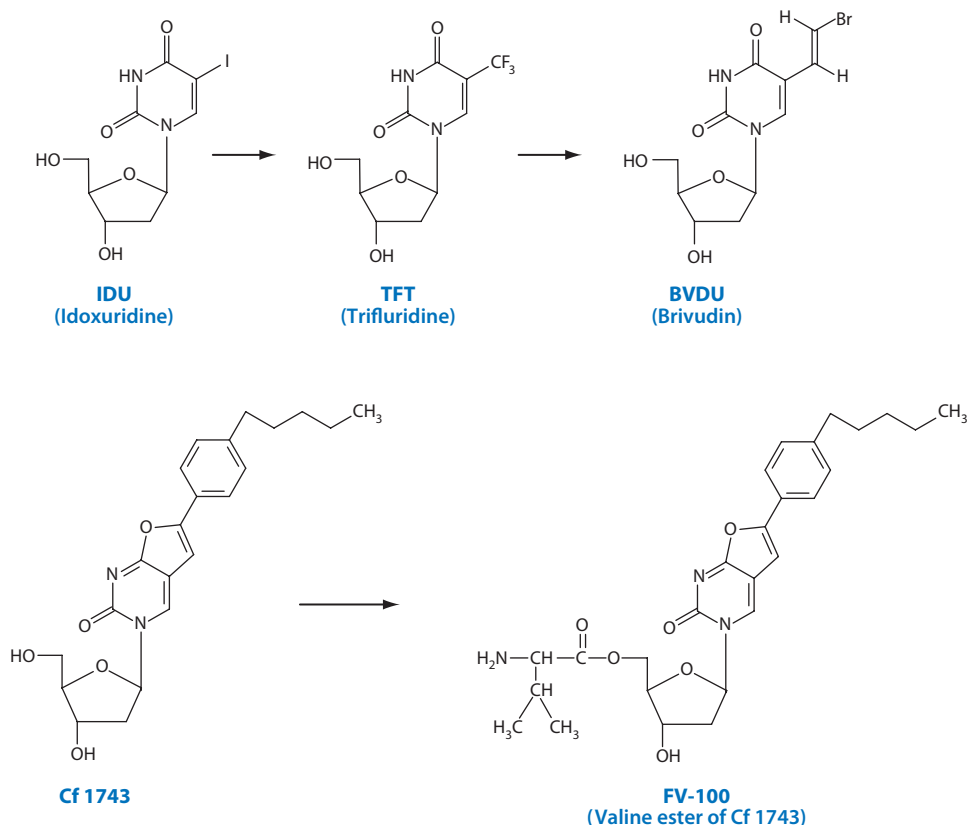


Figure 1

5-Substituted 2'-deoxyuridines and bicyclic furo[2,3-d]pyrimidine nucleoside analogs.

epithelial HSV-1 keratitis (33), and superior to TFT (1%) in the topical treatment of deep stromal HSV-1 keratitis (34) and uveitis (35). BVDU (0.1% eye drops, at 1-h intervals during the day only) was found effective in the treatment of HSV keratitis where other antiviral drugs (e.g., idoxuridine, trifluridine, vidarabine, or acyclovir) had failed to ameliorate the disease before treatment was switched to BVDU (36). Regrettably, despite exhaustive evidence for the efficacy of BVDU in the topical treatment of HSV keratitis, the compound has never been formally developed or licensed for this ophthalmic indication.

Although BVDU is active against VZV at nanomolar concentrations, it can still be surpassed in potency by the bicyclic furo[2,3-d]pyrimidine nucleoside analogs (BCNAs), such as Cf 1743 (37–39). This makes Cf 1743 the most potent anti-VZV compound discovered so far (40). The BCNAs are active only against VZV, not against HSV or any other virus. They owe this activity to a specific phosphorylation by the VZV-induced thymidine kinase (TK) (the BCNAs are inactive against TK-deficient VZV strains). Yet their exact target of action, although believed to be the viral DNA polymerase, still remains to be clarified (41). From a clinical viewpoint, the L-valine ester of Cf 1743 (termed FV-100 for the company FermaVir) has been further developed for the oral treatment of herpes zoster (42); it has recently entered phase II clinical trials.

TK: thymidine kinase

ACYCLOVIR PRODRUGS

When acyclovir [9-(2-hydroxyethoxymethyl)guanine] was first described in 1978 as an antiherpesvirus agent (43), few other antiviral drugs were available (e.g., methisazone, amantadine, idoxuridine, trifluridine, and vidarabine). Its selectivity as an antiherpetic agent had been recognized by Elion et al. (44). In attempts to improve the aqueous solubility of the compound, we synthesized water-soluble esters of acyclovir, i.e., the 2'-*O*-glycyl, 2'-*O*-alanyl, and 2'-*O*-3-carboxypropionyl esters (45). We found that these esters were equally as active as acyclovir in inhibiting HSV-1 and HSV-2 replication in cell culture (45). We judged these esters to be advantageous over acyclovir for two applications: (*a*) as eye drops (instead of ointment) for the treatment of herpetic eye infections, and (*b*) for intramuscular (instead of intravenous bolus) injection. Maudgal et al. (46) then showed 2'-*O*-glycyl acyclovir to be efficacious in the topical treatment of epithelial and stromal HSV keratitis, and the associated iritis, when administered as a 1% eye drop formulation in rabbits.

To increase the oral bioavailability of acyclovir, the L-valine ester prodrug was chosen (47), and valaciclovir would eventually replace acyclovir for the oral treatment of both HSV and VZV infections. The same approach (i.e., L-valine ester) would later be followed to increase the oral bioavailability of ganciclovir (i.e., valganciclovir) and Cf 1743 (i.e., FV-100, see *supra*). For penciclovir, a different approach with the prodrug famciclovir, which is based on the diacetyl ester of 6-deoxypenciclovir, was followed (48). [The conversion of famciclovir to penciclovir thus requires two deacetylations, as well as an oxidation step (the latter may be catalyzed by xanthine oxidase, also shown to convert 6-deoxyacyclovir to acyclovir) (49). Alternatively, the conversion requires aldehyde oxidase, shown to oxidize 6-deoxypenciclovir to penciclovir (50).] Valaciclovir and famciclovir are routinely used for the treatment of HSV and VZV infections, and ganciclovir is used for the treatment of cytomegalovirus (CMV) infections, although it should be recognized that (val)ganciclovir is highly effective against HSV infections as well (see **Figure 2**).

CMV:

cytomegalovirus

AZT: 3'-azido-2',3'-dideoxythymidine

HIV: human immunodeficiency virus

ddN: 2',3'-dideoxynucleoside

d4T: 2',3'-didehydro-2',3'-dideoxythymidine (stavudine)

NRTI: nucleoside reverse transcriptase inhibitor

THE 2',3'-DIDEOXYNUCLEOSIDE ANALOGS

In 1980, we described the antiviral and antimetabolic effects of a number of 2',3'-dideoxynucleoside analogs, including 3'-azido-2',3'-dideoxythymidine (AZT) (51). Human immunodeficiency virus (HIV) was not included in the assays (which did include HSV), simply because neither the disease nor the virus had been identified at the time. AZT was first shown to be an anti-HIV agent (two years before it became the first approved anti-HIV drug) in October 1985 by Mitsuya et al. (52), and by March 1986, Mitsuya & Broder (53) had described several other 2',3'-dideoxynucleosides (ddNs) as anti-HIV agents, including 2',3'-dideoxyinosine (didanosine, or ddI) and 2',3'-dideoxycytidine (zalcitabine, or ddC). [At that time, HIV was termed T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV).] The mechanism of antiviral action of AZT was attributed by Furman et al. (54) to an inhibitory effect of the active 5'-triphosphate metabolite of AZT on the viral reverse transcriptase.

AZT unleashed the search for new ddNs with, it was hoped, equal potency but lower toxicity. Success came quickly with the (almost simultaneous) description of the anti-HIV activity of 2',3'-didehydro-2',3'-dideoxythymidine (stavudine, or d4T) by three groups [our own group in Leuven (55), Prusoff's at Yale (56), and Yamamoto's in Tokyo (57)]. Stavudine would become the fourth and one of the most popular anti-HIV drugs to be used worldwide (58). Then these followed, in chronological order of their approval: the fifth, lamivudine [originally described as its racemic mixture BCH-189 (59)]; the sixth, abacavir (60); and the seventh, emtricitabine [(-)FTC] (61). Thus the current armamentarium of licensed ddNs, now also referred to as nucleoside reverse transcriptase inhibitors (NRTIs), includes seven licensed anti-HIV drugs (62, 63) (see **Figure 3**).

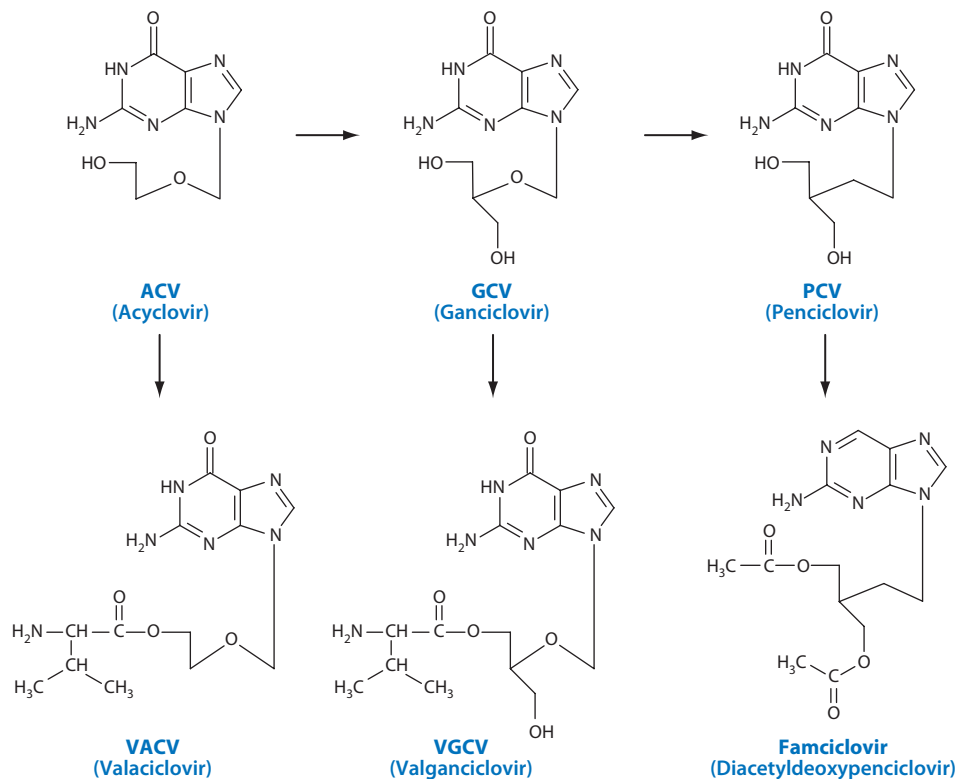


Figure 2

Acyclic guanosine analogs and their prodrugs.

The present NRTI development pipeline contains a wealth of ddNs in various stages of clinical or preclinical development, such as apricitabine, elvucitabine (Ld4FC), amdoxovir (DAPD), alovudine (FLT), racivir [(±)FTC], festinavir (4'-Ed4T), and several others (64). For some of these compounds (e.g., FLT), the roots date back to the 1980s when Herdewijn et al. (65) described the anti-HIV activity of a variety of 3'-fluoro-2',3'-dideoxynucleoside analogs. For other compounds (e.g., emtricitabine, amdoxovir, apricitabine, and racivir), the thiacytidine BCH-189, synthesized by the late Bernard Belleau (59), may well be viewed as the starting point.

THE ACYCLIC NUCLEOSIDE PHOSPHONATES

The “Old” Acyclic Nucleosides

The origin of the acyclic nucleoside phosphonates (ANPs) (66) dates back to May 1976, when Antonin Holý and I met for the first time at the Symposium on Synthetic Nucleosides, Nucleotides, and Polynucleotides in Göttingen, Germany, and started our collaboration on the antiviral activity of nucleoside analogs. This collaboration quickly led to the identification of (S)-DHPA [(S)-9-(2,3-dihydroxypropyl)adenine] as an aliphatic nucleoside analog with broad-spectrum antiviral properties (67). This was a few months after the specific anti-HSV activity of acyclovir had been reported by Elion et al. (44) and Schaeffer et al. (43). Unlike the mechanism of action of acyclovir, which could promptly be attributed to a specific phosphorylation by the herpes viral TK (44), the

ANPs: acyclic nucleoside phosphonates

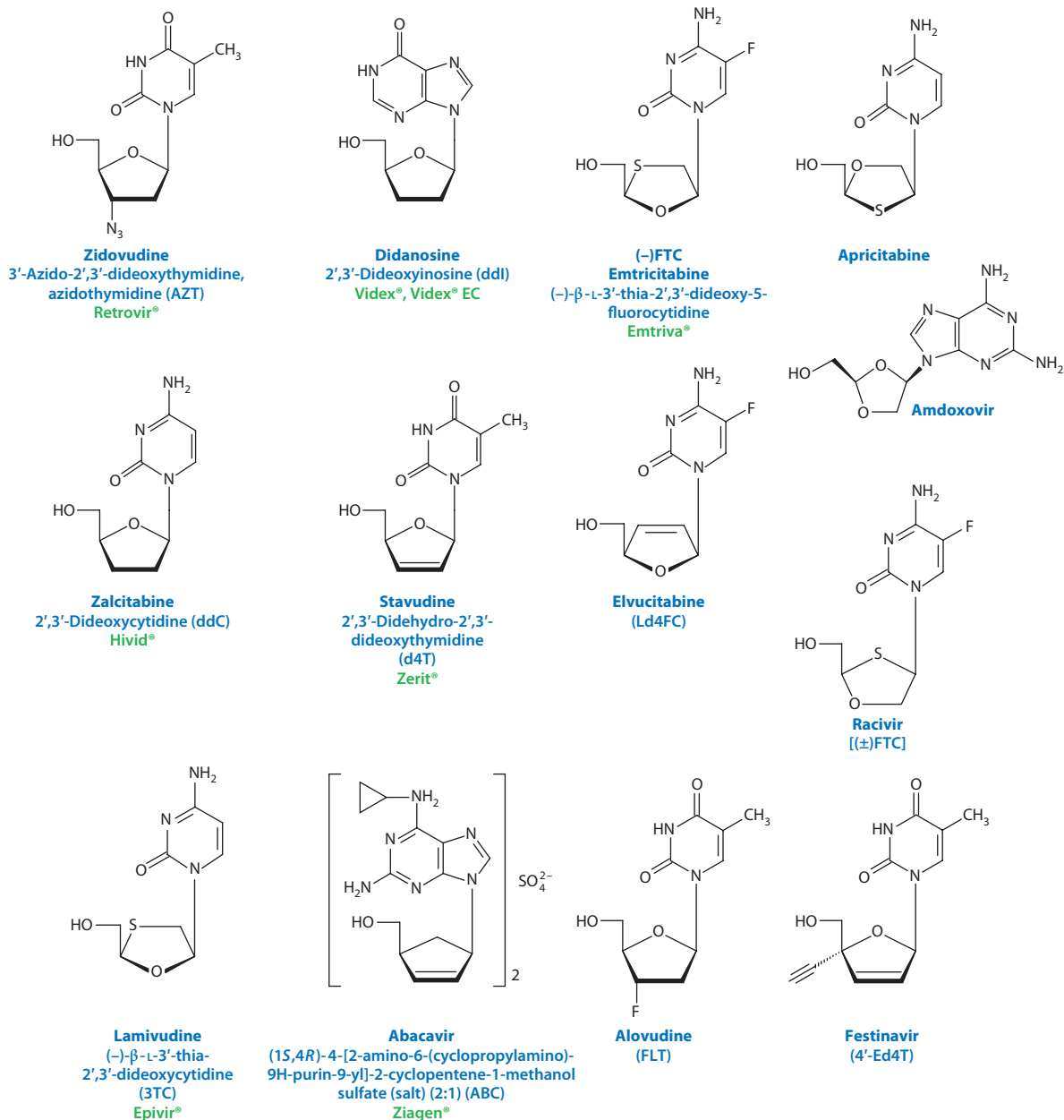


Figure 3

Nucleoside reverse transcriptase inhibitors (NRTIs).

mechanism of action of (S)-DHPA was at first unclear. It would be later attributed to an interaction with the S-adenosyl-L-homocysteine hydrolase (68, 69).

The era of the ANPs (**Figure 4**) (70) started in 1986 with the description of (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine [(S)-HPMPA] as a novel, selective, broad-spectrum anti-DNA virus agent (71). This was followed soon thereafter, in 1987, by the description of the broad

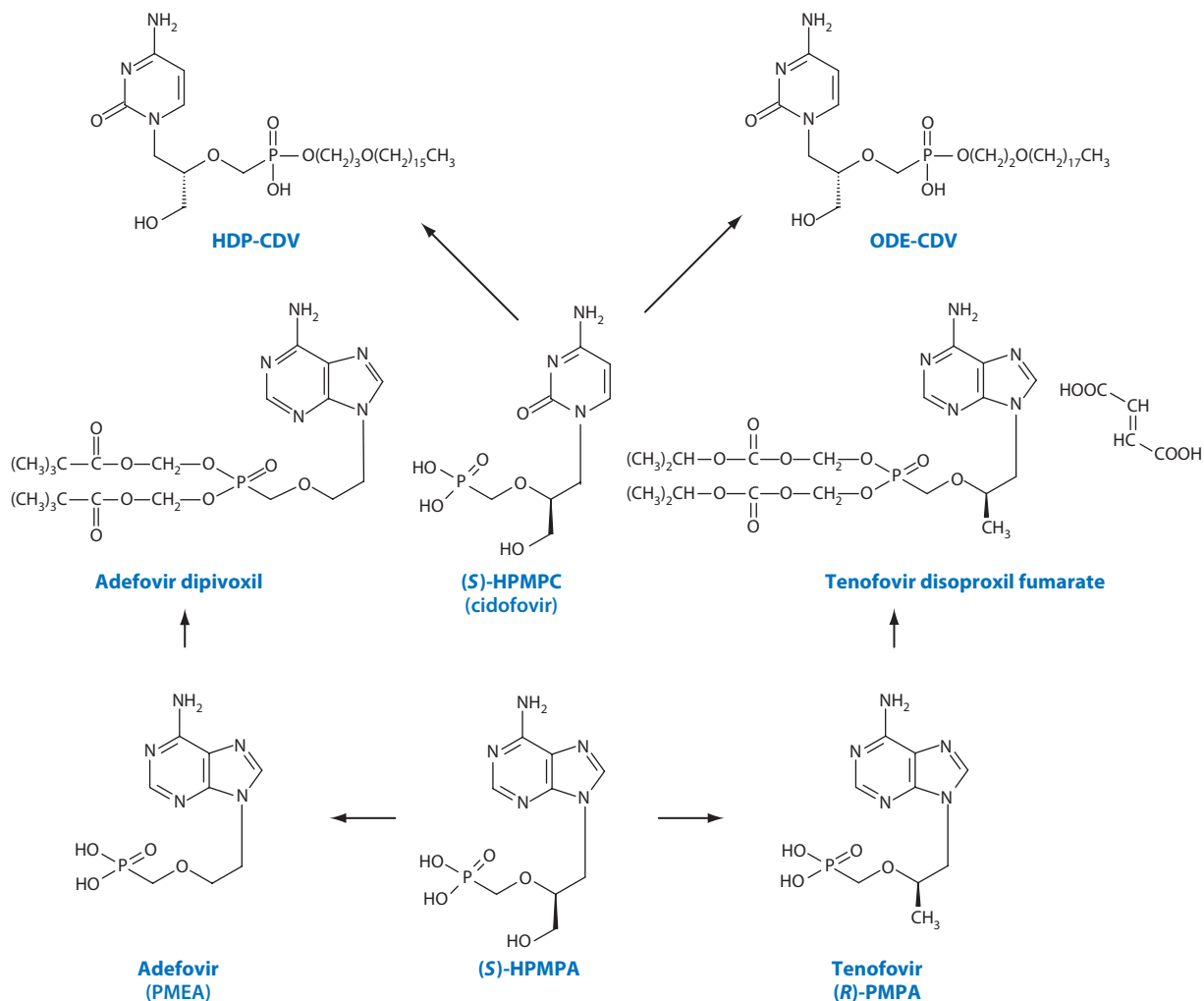


Figure 4

“Old” acyclic nucleoside phosphonates (ANPs). Abbreviations: HDP-CDV, hexadecyloxypropyl derivative of cidofovir; ODE-CDV, octadecyloxyethyl derivative of cidofovir.

anti-DNA virus activity of (S)-HPMPC and several other phosphonylmethoxyalkyl derivatives of purines and pyrimidines (72). (S)-HPMPC (cidofovir) would eventually be licensed for clinical use (in 1996) for the intravenous treatment of CMV retinitis in AIDS patients (73). The incidence of CMV infections such as retinitis has recently dropped drastically thanks to the successful treatment of AIDS with the current anti-HIV drugs. Yet the broad anti-DNA virus activity of (S)-HPMPC, similar to that of (S)-HPMPA (71), makes the compound potentially useful for the treatment of various DNA virus infections, poxvirus infections (74), and papilloma and polyoma virus infections (75, 76). (S)-HPMPC would be more effective than smallpox vaccination against a lethal monkeypox virus infection (77).

The problem with cidofovir, however, is that it has to be administered intravenously (or intratumorally) at a dosage not exceeding 5 mg kg⁻¹ weekly or every two weeks, so as not to exceed

the nephrotoxicity threshold. To increase cidofovir's oral bioavailability and at the same time reduce its nephrotoxicity, several alkoxyalkyl derivatives have been prepared, the most prominent of which are octadecyloxyethyl (ODE) and hexadecyloxypropyl (HDP) (41, 78). The ODE and HDP derivatives of (S)-HPMPC [or (S)-HPMPA] should, in principle, be applicable in the oral treatment of all infections due to viruses sensitive to (S)-HPMPC or (S)-HPMPA, such as herpes, adeno-, pox-, polyoma-, and papillomavirus infections.

Simultaneously with (S)-HPMPA (71, 72), we described PMEAs as specific antiretroviral agents, and the anti-HIV activity of PMEAs (adefovir) was further documented by Pauwels et al. (79). Because adefovir, like cidofovir, has low oral bioavailability, its bis(pivaloyloxymethyl) ester (adefovir dipivoxil) was further developed (80, 81). Adefovir dipivoxil was originally pursued as a potential anti-HIV drug, but it was eventually abandoned for this purpose for a number of reasons: (a) At the dosage (125 mg daily) needed to be effective against HIV, it proved nephrotoxic upon prolonged administration (>6 months); (b) tenofovir, which had just come along, proved at least as potent against HIV and clearly less nephrotoxic; and (c) adefovir, going back to the original observations of Yokota et al. (82, 83) and Heijntik et al. (84, 85), turned out to be effective against hepatitis B virus (HBV) at a much lower dose (10 mg daily) than was needed for HIV. Thus adefovir dipivoxil was successfully launched at a dose of 10 mg per day for the treatment of HBV (86, 87). In later studies, Marcellin et al. (88) would demonstrate that tenofovir disoproxil fumarate was more effective in the treatment of HBV than adefovir dipivoxil. This is obviously not surprising, considering the difference in dosage used (300 mg versus 10 mg, daily). (In vitro, adefovir and tenofovir are approximately equipotent in inhibiting the replication of HBV.) What is more surprising, however, is that adefovir dipivoxil at a dosage of 10 mg had such a dramatic effect on the HBV titer ($\sim 4 \log_{10}$ drop), as convincingly shown in a crossover study by Hadziyannis et al. (89). This raises the question as to whether the antiviral effectiveness of adefovir against HBV may be enhanced by a preferential uptake by the liver and/or additional immunoregulatory effects.

Following up on (S)-HPMPC and PMEAs as the lead compounds (71), we described in 1991 the 9-(3-fluoro-2-phosphonylmethoxypropyl)purines (90) and in 1993 the (R)-9-(2-phosphonylmethoxypropyl)purine derivatives (91) as potent and selective antiretroviral agents. The latter publication corresponded to the first description of the anti-HIV activity of (R)-PMPA, later to become known as the aforementioned tenofovir and currently a cornerstone (as tenofovir disoproxil fumarate) in the treatment of HIV infections (licensed in 2001) as well as HBV infections (approved in 2008). It is curious as to why (R)-PMPA, and not its more potent congener, the 2,6-diaminopurine counterpart (R)-PMPDAP, was further developed. This may be related to the perception of John C. Martin (currently chairman of the board and chief executive officer at Gilead) that, in contrast with adenine (6-aminopurine) and guanine, the 2,6-diaminopurine represents an "unnatural" purine, and that there might be a good reason (risk for mutations?) that nature had selected adenine and guanine and not 2,6-diaminopurine for building up the normal base sequence in RNA and DNA (J.C. Martin, personal communication).

Ultimately, (R)-PMPA—and not (R)-PMPDAP [both compounds are much more active than their (S)-enantiomers]—was selected for further development, and because of its poor oral absorption, (R)-PMPA was converted into its oral prodrug, bis(isopropylloxycarbonyloxymethyl) (R)-PMPA (92, 93) (tenofovir disoproxil). The latter was subsequently formulated as tenofovir disoproxil fumarate, to be launched in 2001 for the treatment of HIV infections (94).

The "New" Acyclic Nucleoside Analogs

In recent years, several new acyclic (and cyclic) nucleoside phosphonates have been described for their anti(retro)viral activity (**Figure 5**), namely 6-[2-(phosphonomethoxy)alkoxy]-2,4-diaminopyrimidines [e.g., (R)-HPMPO-DAPy, PMEO-DAPy, (R)-PMPO-DAPy, and

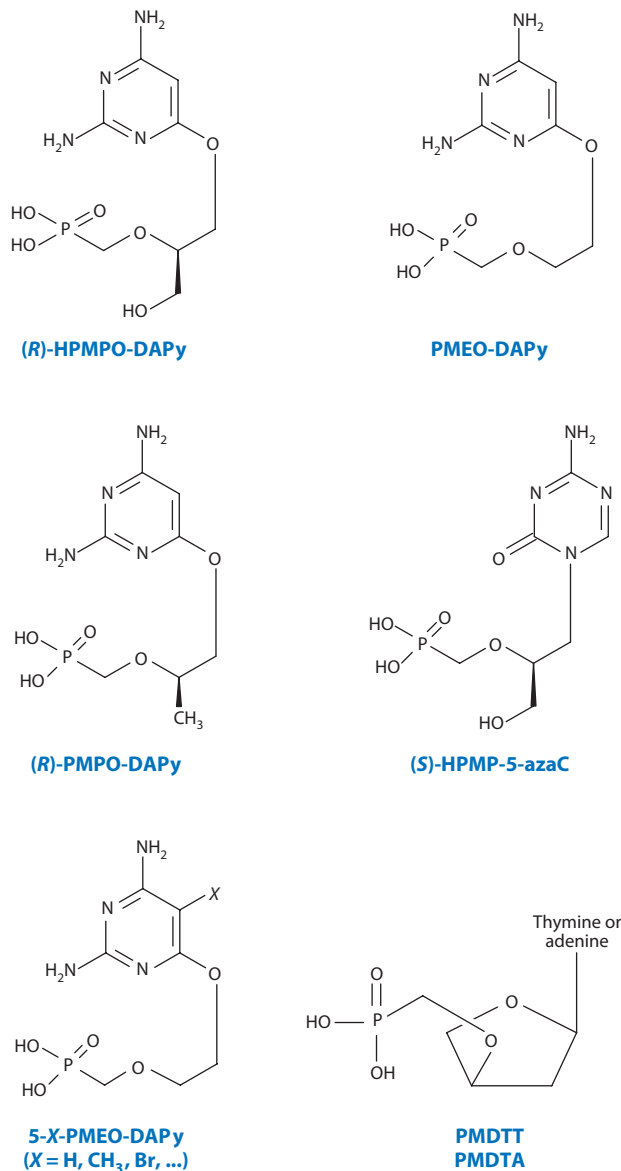


Figure 5

“New” acyclic (and cyclic) nucleoside phosphonates.

5-substituted PMEO-DAPys] (95–97), deoxythreosyl phosphonate nucleosides [e.g., PMDTA and PMDTT (98)], and triazine analogs derived from cidofovir [e.g., (S)-HPMP-5-azaC (99) and the ester prodrugs thereof (100)]. (R)-HPMPO-DAPy and (S)-HPMP-5-azaC (and ester prodrugs thereof) yield therapeutic potential against viruses that are sensitive to (S)-HPMPC (cidofovir), such as polyoma-, papilloma-, adeno-, herpes, and poxvirus. (For poxvirus, see, for example, References 101–103.) (R)-PMPO-DAPy and PMEO-DAPy (and 5-substituted derivatives thereof) may yield particular potential as antiretroviral (i.e., anti-HIV and/or anti-HBV) agents (104, 105).

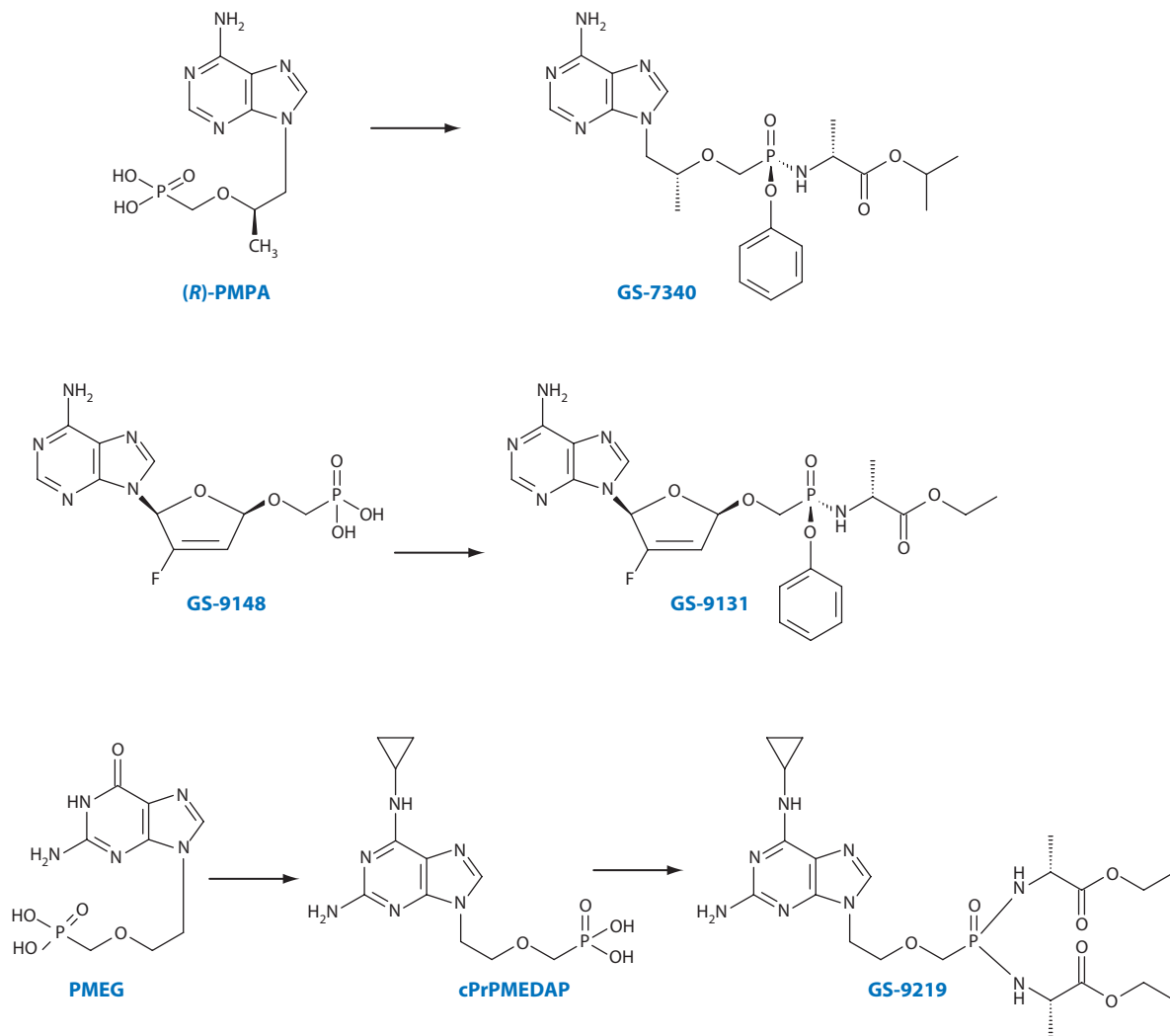


Figure 6

Phosphonoamidates of acyclic (and cyclic) nucleoside phosphonates.

The new ANPs, such as (*S*)-HPMP-5-azaC (106), may or may not have distinct advantages over the established ones (cidofovir, adefovir, and tenofovir), but unless their potential efficacy against the various viruses falling within their specific spectrum of activity is thoroughly evaluated, we will never find out about their potential usefulness.

The Acyclic (and Cyclic) Nucleoside Phosphonoamidates

Conversion of tenofovir to its isopropylalaninyl phosphonoamidate phenyl ester prodrug (GS-7340) was shown by Bill Lee and colleagues (107) to specifically target tenofovir to the lymphatic tissue (see **Figure 6**). This propensity of phosphonoamidate prodrugs for the lymphoid cells has also been demonstrated with GS-9131, the phosphonoamidate prodrug of GS-9148

NNRTI: non-nucleoside reverse transcriptase inhibitor

(the 2'-FD4A phosphonate) (108, 109). GS-9148, akin to the acyclic nucleoside phosphonate analogs (ANPs), is a substrate for renal transporters hOAT1 and hOAT3 and the multidrug resistance protein 4 (MRP4). However, in contrast with the ANPs, GS-9148 would have a limited renal accumulation and thus low nephrotoxicity (110), which would be an additional assurance of safety in patients treated with GS-9148's prodrug GS-9131. Still following the same principle, the bis(phosphonoamidate) GS-9219 was constructed as a prodrug of 9-(2-phosphonomethoxyethyl)-N⁶-cyclopropyl-2,6-diaminopurine (cPrPMEDAP), itself a prodrug of the cytotoxic agent 9-(2-phosphonomethoxyethyl)guanine (PMEG), first described in 1987 when the many other ANPs were first described (72). The antitumor potential of cPrPMEDAP was first described in 1999 by Hatse et al. (111) and Naesens et al. (112). The phosphonoamidate derivatization, as in GS-9219, targets cPrPMEDAP to the lymphoid cells. In this sense, GS-9219 acts as a proprodrug of PMEG, its final cytostatic action mediated by the 5'-triphosphate of PMEG. GS-9219 has been shown to cause a remarkable regression of spontaneous, advanced non-Hodgkin's lymphoma in beagle dogs (113) and is now in clinical trials (phase I/II) in patients with hematological malignancies (113).

THE NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

The term NNRTIs was coined in the mid-1990s to distinguish non-nucleoside reverse transcriptase inhibitors (all of which interact with an allosteric site of the HIV-1 reverse transcriptase and, accordingly, inhibit HIV-1 but not HIV-2 replication) from the aforementioned NRTIs (zidovudine, didanosine, zalcitabine, and so on) (114–117). As I reviewed previously (118), the era of the NNRTIs started with the discovery in our laboratory of two series of compounds: the 1-(2-hydroxyethoxymethyl)-6-(phenylthio)thymine (HEPT) derivatives (119, 120) and the 4,5,6,7-tetrahydro-imidazo[4,5,1-*jk*][1,4]benzodiazepine-2(1*H*)-one (TIBO) derivatives (121, 122). The HEPT and TIBO derivatives were discovered in totally different ways, independently from each other (see **Figure 7**).

The original HEPT compound (code no. TS-II-25) was forwarded to me from Birmingham, United Kingdom, by the late Dr. R.T. Walker on behalf of Hiromichi Tanaka from Showa University (Tokyo, Japan) with the request to test the compound against HSV. Obviously, this question was inspired by the presence of the same 2-hydroxyethoxymethyl side chain in TS-II-25 that was present in acyclovir, but at that time we knew already that such a side chain, when attached to a pyrimidine moiety, did not result in any activity against HSV; as expected, TS-II-25 was totally inactive against HSV. This should have been the end of the story, but in those days (August–September 1986), Masanori Baba (now a professor at Kagoshima University in Japan) had just found d4T (stavudine) to be highly active when using MT-4 cells (55), and I asked him to check whether, by any chance, any of the Showa University compounds displayed any activity against HIV. Surprisingly, TS-II-25 did. This observation (originally dating from 1987) was quite puzzling (at first, I thought the result might have arisen from a contamination of the sample with AZT). Two years later, we ascertained that TS-II-25 directly interacted with an allosteric site of the HIV-1 reverse transcriptase that was distinct from, but spatially and functionally related with, the substrate binding site (123, 124). Further lead optimization led to the identification of MKC-442 (emivirine) (125) as the clinical drug candidate, but Mitsubishi Kasei Corporation (MKC), the owners of the compound, were concerned about possible drug resistance development. By the time this concern was alleviated (the compound was in the meantime transferred from MKC to Triangle Pharmaceuticals) (126), the landscape, with three NNRTIs on the market (nevirapine, delavirdine, and efavirenz), had become too competitive, and further development of emivirine was stopped.

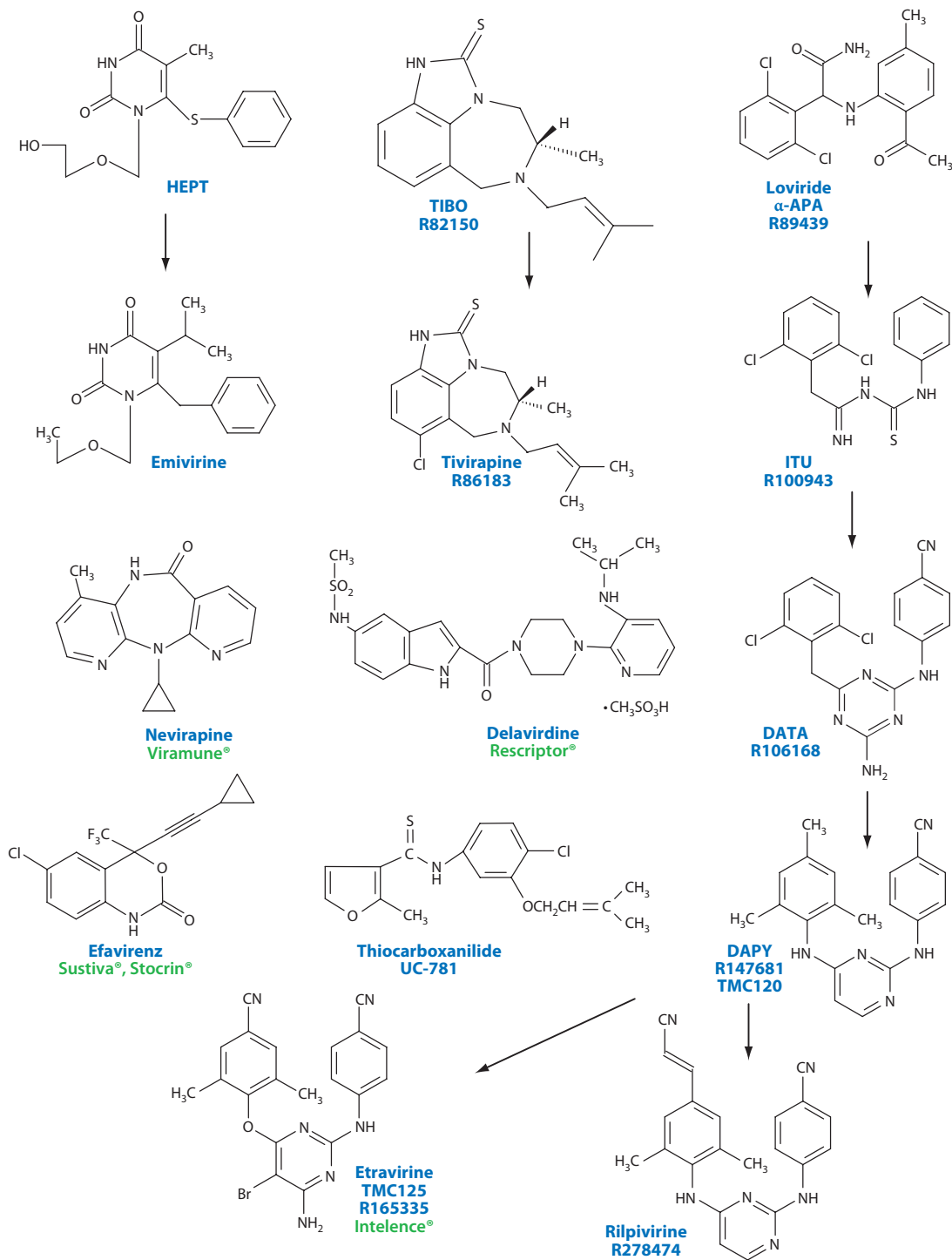


Figure 7

Non-nucleoside reverse transcriptase inhibitors (NNRTIs).

The discovery of the TIBO derivatives followed a totally different path. It started with a long personal meeting I had on November 5, 1986, in Beerse with the late Dr. Paul Janssen, when we agreed to join the forces of the Janssen Research Foundation (Janssen Pharmaceutica) and our laboratory at the Rega Institute to find a “cure” for AIDS. At Janssen they had the necessary chemical know-how, and in our laboratory Rudi Pauwels had worked out a rapid and automated system, a tetrazolium-based colorimetric assay, for evaluating a virtually unlimited number of compounds for their anti-HIV activity [this method was first described in *the Journal of Virological Methods* (127)]. This system has become, worldwide, the most widely used assay for detecting HIV replication inhibitors (128).

Starting in July 1987, after a formal agreement was signed between the Janssen Research Foundation and the Katholieke Universiteit Leuven, we started with the rational evaluation of approximately 600 compounds from the Janssen library. Within two years, we had identified several lead compounds—one of which was TIBO R82510 (121)—as new specific HIV-1 inhibitors that interact with the HIV-1 reverse transcriptase (121, 122). This breakthrough, published in the February 1, 1990, issue of *Nature*, could be heralded as the first publication describing the NNRTI concept. However, the chemical synthesis of TIBO R82510 and the clinical drug candidate derived thereof, R86183, proved to be too cumbersome (some 11 steps!), so the attention from Janssen shifted to simpler scaffolds with equally potent and selective anti-HIV-1 activity. Thus α -anilino-phenylacetamide (α -APA) was identified as the next (potential) anti-HIV drug candidate (129). As α -APA (loviride) did not fulfill the desired pharmacokinetic requirements (part of the problem was that loviride, given its chiral structure, actually forms a racemic mixture), new derivatives were synthesized, e.g., iminothiourea (ITU) (130), diaryl triazine (DATA) (131), and diaryl pyrimidine (DAPY) analogs (132). The DAPY family yielded three congeners: dapivirine (TMC120), still under development as a topical microbicide to prevent HIV infections; etravirine (TMC125) (133), which has been licensed (Intelence®); and rilpivirine (R278474, TMC278) (134), which can be considered Dr. Paul Janssen’s final legacy. Rilpivirine (135) fulfills the requirements (134) expected from an “ideal” anti-HIV drug (i.e., high potency and selectivity, high oral bioavailability, minimal side effects, and easy synthesis). It would come close to Dr. Paul Janssen’s dream to find a “cure” for AIDS (136).

DRUG COMBINATION THERAPY

For the same reasons that account for the combination of antitubercular drugs in the treatment of tuberculosis [(a) to obtain synergistic activity, (b) to reduce the individual drug dose levels (and associated drug toxicity), and (c) to reduce the risk of drug resistance development], anti-HIV drug policy is similarly based on drug combination regimens. Considering the different classes of anti-HIV drugs currently available [NRTIs, nucleotide reverse transcriptase inhibitors (NtRTIs), NNRTIs, protease inhibitors (PIs), fusion inhibitors (FIs), coreceptor inhibitors (CRIs), and integrase inhibitors (INIs)] (62, 63), the number of possible multidrug combinations is unrealistically high (**Figure 8**). Yet the number of approved fixed-dose drug combinations is rather limited; the only one containing three different classes of compounds is Atripla®, which consists of 300 mg TDF (tenofovir disoproxil fumarate), 200 mg emtricitabine [(–)FTC], and 600 mg efavirenz. Atripla (available since 2006) is meant to be taken as a single pill once daily. (The combination of TDF with emtricitabine has also been available since 2004 as Truvada®.) Currently, more than two-thirds of the U.S. patients treated with antiretrovirals are receiving TDF (Viread®), or TDF in combination with emtricitabine (Truvada), or TDF in combination with both emtricitabine and efavirenz (Atripla).

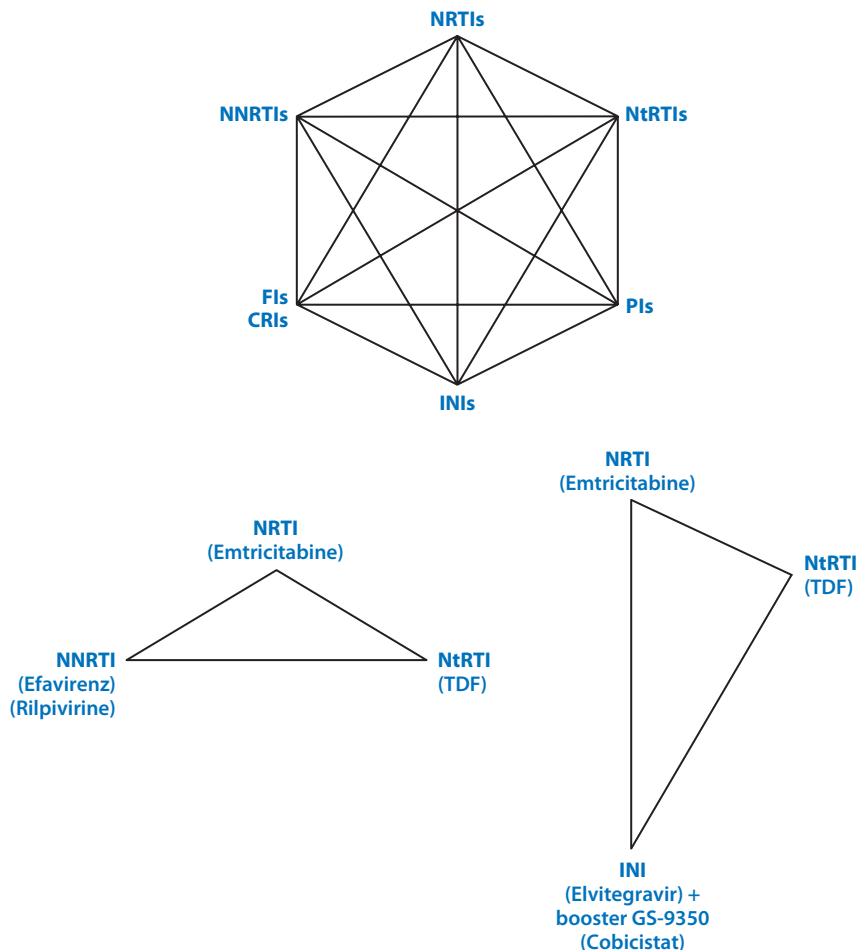


Figure 8

Combination of anti-HIV drugs. Acronyms: CRI, coreceptor inhibitor; FI, fusion inhibitor; INI, integrase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NtRTI, nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir disoproxil fumarate.

In the future, new fixed-dose, triple-drug regimens may be based on single (daily) pills containing TDF (300 mg), (–)FTC (200 mg), and rilpivirine (25 mg). Another multidrug combination pill may be based on the combination of TDF (300 mg), (–)FTC (200 mg), and the new INI elvitegravir (125 mg) together with a booster (“enhancer”) thereof, GS-9350 (125 mg). Another successful combination is based on the use of raltegravir in combination with zidovudine and lamivudine (137, 138). The inconvenience of raltegravir, similar to that of Combivir® (zidovudine plus lamivudine), is that it has to be administered bid (twice daily), whereas elvitegravir can be given qd (once daily). According to Walensky et al. (139), and quoted by Marty Hirsch (140), “the use of antiretroviral drugs may have saved 3 million years of life (which compares favorably with many other interventions for chronic diseases).”

A very important role may be expected for TDF (Viread) or any of its combination products in a preemptive strike against HIV (141): its use for the pre-exposure prophylaxis (PrEP) of HIV

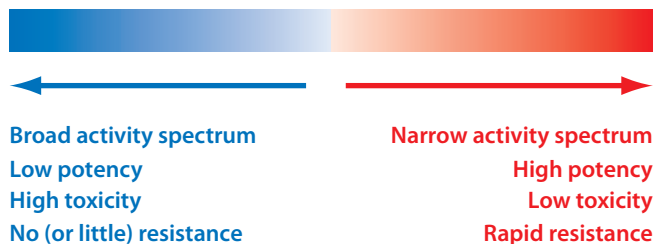


Figure 9

Gradient in antiviral drug parameters.

infections (75). A number of PrEP trials enrolling approximately 20,000 individuals have been initiated in the United States, South America, southern Africa, eastern Africa, and Asia (Thailand) with either Truvada or Viread (141). There are solid experimental arguments for the prophylactic effectiveness of tenofovir against simian immunodeficiency virus (SIV) infections in monkeys, irrespective of the route of transmission [parenteral (142), sexual (intravaginal) (143), or perinatal (144)]. The effectiveness and safety of a vaginal tenofovir gel formulation for the prevention of HIV infection in women has been recently demonstrated (145).

CONCLUDING REMARKS

For the treatment of HIV infections, drug combinations (originally termed HAART, for highly active antiretroviral therapy) have become a standard procedure. This contrasts with the treatment of other virus infections, such as herpes (HSV, VZV, or CMV), in which treatment is routinely based on the use of a single antiviral drug [(val)acyclovir for HSV and VZV, and (val)ganciclovir for CMV, for example]. Also, for the treatment of influenza virus infections, single drugs are generally used, i.e., neuraminidase inhibitors [oseltamivir or zanamivir (146, 147)].

Despite the similarity of HBV to HIV, drug combination is generally not implemented for the treatment of HBV infections, for which six compounds have been licensed: interferon, lamivudine, adefovir dipivoxil, entecavir, telbivudine, and, most recently (88), tenofovir disoproxil fumarate. For the treatment of hepatitis C virus (HCV) infections, the current standard care involves pegylated interferon combined with ribavirin (148). It is likely that in the future, the drug regimens for HCV infections will be extended to include both HCV protease inhibitors and/or HCV RNA polymerase inhibitors. Similar to NRTIs and NNRTIs for HIV infections, both nucleoside and non-nucleoside types of HCV RNA polymerase inhibitors may be anticipated in the future (149).

Conceptually (**Figure 9**), antiviral drugs may be viewed as a compromise between the two extremes: at one side of the gradient, compounds have a broad activity spectrum, high toxicity, and low potency, but no risk for resistance; at the other side of the gradient, compounds have a narrow activity spectrum, high potency, and low or no toxicity, but high risk for resistance development. Individual compounds fall somewhere between the two extremes, and drug combinations will likely result in an intermediary or mixed response.

EPILOGUE

This review aimed at describing the often long and convoluted route of development of a number of antiviral drugs that finally made a successful appearance on the antiviral drug market (e.g., BVDU, valaciclovir, cidofovir, adefovir, tenofovir) or should do so soon (e.g., FV-100, rilpivirine). In one particular case, however, the antiviral drug AMD3100 did not follow the foreseen path

and was finally developed as a stem cell mobilizer (150, 151); it is used for transplantation of hematopoietic stem cells in patients with hematological malignancies such as non-Hodgkin's lymphoma or multiple myeloma. This means that in drug design, discovery, and development, ample opportunity should be left to serendipity and to perseverance in exploring the unexpected.

DISCLOSURE STATEMENT

The author is linked as coinventor to several compounds described here, such as BVDU (brivudin), BCNAs (i.e., Cf 1743), amino acyl esters of acyclovir, HEPT derivatives, and acyclic nucleoside phosphonates (ANPs cidofovir, adefovir, and tenofovir). He is a consultant for Gilead Sciences and is entitled to receive royalties through an arrangement between Gilead Sciences and the University of Leuven.

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